

사람 치주인대줄기세포의 교원질 형성에 대한 법랑기질 유도체의 영향

The Effect of Enamel Matrix Derivatives on the Collagen Formation by Human Periodontal Ligament Stem Cells both in vitro and in vivo Analysis

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ABSTRACT

사람 치주인대줄기세포의 교원질 형성에 대한 법랑기질 유도체의 영향

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목적: 법랑기질 유도체(EMD)가 사람 치주인대 줄기세포(hPDLSC)의 조직 형성에 미치는 영향을 in vitro와 in vivo 분석 모델을 이용해 평가한다.

재료 및 방법: hPDLSC를 배양하여 운반체와 함께 면역 억제된 쥐 등에 이식하였다; (1)대조군: EMD 처리하지 않은 운반체에 심어진 hPDLSC 군 (EMD-/hPDLSC*), (2)실험군: EMD 처리한 운반체에 심어진 hPDLSC군 (EMD+/hPDLSC*). 각 군당 5마리씩 시행하고 8주 후 희생하였다. 조직학적, 조직계측학적 분석을 통해 형성된 백악질의 면적과 백악세포의 수 그리고 사피 섬유유 수를 계측하였으며 면역조직화학적 분석을 통해 백악질과 교원질 형성을 평가하였다. 또한 in vitro에서 hPDLSC의 수용성 교원질과 glycosaminoglycan 형성에 대한 EMD의 효과를 분석하였다.

결과: 조직학적 분석에서 교원질성 치주 인대 조직이 실험군에서 현저하게 많이 생성된 것을 관찰할 수 있었다. 형성된 백악질의 면적과 백악세포의 수는 군 간 차이가 없었으나, 새롭게 형성된 사피 섬유유 수의 수는 실험군에서 대조군보다 유의하게 많았다($p<0.05$). 교원질 형성에 대한 면역조직화학적 분석 결과, 실험군에서 I, III형 교원질과 hydroxyproline의 발현이 높았다. 또한 in vitro에서 hPDLSC에 의한 수용성 교원질과 glycosaminoglycan 형성이 EMD의 농도에 비례하여 증가하였다 ($p<0.05$).

결론: EMD는 hPDLSC에 의한 사피 섬유 및 교원질 생성을 증가시키고, 이는 새로운 백악질의 기능적 부착과 치주조직 재생에 중요한 역할을 한다.

Key words : enamel matrix proteins, mesenchymal stromal cells, periodontal ligament, periodontium, regeneration, tissue engineering

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I. Introduction

Enamel matrix derivatives(EMD) are extracts of porcine enamel matrix proteins secreted during the development of tooth-supporting tissues. Emdogain(Institute Straumann, Basel, Switzerland), a commercialized EMD product introduced in 1997 by Hammarstrom, has been used as the one of the current clinical treatment for periodontal tissue regeneration^{1, 2)}. The desired outcome from the clinical application of Emdogain in adult periodontal defect is tissue regeneration that mimics the natural process of tissue development³⁾.

A number of researches have been reported that the application of EMD yielded positive results to conventional periodontal treatment in various type of defect in animal model including intrabony, furcation or dehiscence defects^{2, 4~6)}. They demonstrated that clinical attachment level and radiographic bone fill were enhanced, especially the histological analysis has clearly shown that EMD promotes the regeneration of cementum, alveolar bone, and periodontal ligament following application of EMD. Along with the preclinical studies, the positive effect of EMD also has been represented in a number of clinical studies showing comparable results with guided tissue regeneration(GTR)⁷⁾. Moreover, the use of EMD would be favored by clinicians due to low risk of clinical complication shown in

the GTR technique.

Despite these promising clinical achievements, the precise cellular mechanism underlying the effect of EMD has not been clarified yet. The possible rationale of EMD for periodontal regeneration is that active ingredient such as amelogenin which plays important role during development would stimulate the progenitor to differentiate into cementoblast, osteoblast and collagen producing fibroblast. Diverse in vitro studies have demonstrated that EMD and amelogenins stimulate the growth of multiple mesenchymal cell types, including fibroblasts, cementoblasts, osteoblasts, and periodontal ligament stem cells(PDLSCs)^{8~10)}. Also, it was reported that the epithelial and connective tissue down growth was inhibited by the EMD which determine the attachment gain in histologic analysis¹¹⁾. The EMD may help the periodontal ligament(PDL) cell to be attached with diseased root surface^{3, 12)}. When the PDL cells were treated with EMD, the increased collagen formation could be observed as well as the enhanced osteogenic differentiation potential, suggesting that the EMD also have an effect on the new bone and new cementum formation²⁾. While a number of basic researches on the effect of EMD, the precise cellular mechanism underlying the effect of EMD on PDLSCs for periodontal regeneration still remains unclear.

Regeneration of the Sharpey's fiber in PDL should be considered as one of the critical point for the periodontal regeneration. It was reported that the human PDLSCs(hPDLSCs) could produce not only a new cementum on the matrix, but also a collagenous ligament fiber resembling Sharpey's fiber which was responsible for attachment between the cementum and bone¹³⁾. Although the collagenous ligament fiber formation by PDLSCs has been known accomplishing critical role of attachment, there is lack of information on the effects of EMD on collagenous ligament fiber formation by PDLSCs.

In our previous studies, we have utilized an ectopic transplantation model which has been a straightforward and effective tool to investigate the in vivo behaviors of stem cells of interest. By this model, the sequence of regeneration by hPDLSc could be successfully evaluated in the previous study¹³⁾. Thus, we assumed that the application of EMD to the current study model will help toward gaining a deeper understanding of the effect of EMD on hPDLSCs.

The purpose of the present study was thus to determine the effect of EMD on the tissue forming activity of hPDLSCs, especially the collagenous periodontal ligament formation which is critical composition of periodontal attachment, using in vitro and in vivo analysis models.

II. Materials and Methods

1. hPDLSCs culture

hPDLSCs were isolated and cultured according to a modification of previously reported protocols¹⁴⁾. The experimental protocol was approved by the Institutional Review Board of Yonsei university(2-2010-0016). The subjects were provided informed consent to participate. Four healthy teeth were extracted for orthodontic purposes from one healthy patient(female, 28 years old). hPDLSCs were isolated from the root surface of the 4 extracted premolars using a scalpel, and then cut into pieces under 1 mm diameter.

The small pieces of tissue were digested four or five times at 20-min intervals in α -minimum essential medium(α -MEM; GIBCO, Grand Island, NY, USA) containing 3 mg/ml collagenase type I(WAKO, Tokyo, Japan) and 4 mg/ml dispase(GIBCO) at 37°C. Single-cell suspensions were obtained by passing the mixture through a strainer with a pore size of 70 μ m(Falcon, BD Labware, Franklin Lakes, NJ, USA), and the cells(5×10^5) were seeded on to T75 cell culture dishes containing α -MEM supplemented with 15% fetal bovine serum(GIBCO), 100 μ mL-ascorbic acid 2-phosphate(Sigma-Aldrich, St. Louis, MO, USA), 2 mM-L-glutamine(GIBCO), 100 U/ml penicillin, and 100 μ g /ml streptomycin(GIBCO), and incubated at 37°C in an atmosphere containing 5% CO₂. The third or fifth passage of cells was used for the study. Various experiments including in vitro and in vivo assay were conducted to confirm whether these group of cells possessed

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the characteristics of mesenchymal stem cells.

2. hPDLSC transplantation into an ectopic subcutaneous transplantation model using a carrier treated with or without EMD

Hydroxyapatite/ β -tricalcium phosphate(HA/ β -TCP) powder 80 mg[macroporous biphasic calcium phosphate(MBCP); Biomatlante, Vigneux, France) was used as a carrier for the hPDLSCs. The animals were treated under the following two experimental conditions: (1) hPDLSCs seeded onto an untreated MBCP carrier(EMD/hPDLSC⁺ group), and (2) hPDLSCs seeded onto an EMD-pretreated MBCP carrier(EMD⁺/hPDLSC⁺ group). The results from the negative control group with MBCP are not shown in this study since the results are previously reported in our previous studies^{13, 15, 16}. Carrier was either pretreated or not with EMD, by soaking MBCP carriers overnight with Emdogain gel at a concentration of 30 mg/ml. The mice were treated using a protocol that was approved by the Animal Care and Use Committee, Yonsei Medical Center, Seoul, Korea. hPDLSCs (6×10^6 per carrier) were pre cultured for 1.5 h with the carrier at 37°C in a 5% CO₂ atmosphere before transplantation. The carriers were loaded subcutaneously on each side(left and right, respectively) into the dorsal region of 5-week-old male CB17 severe combined immunodeficiency(SCID) mice(n=5 per group, with two ectopic transplantations in each animal). The animals were allowed to heal

for 8 weeks, and then they were sacrificed.

3. Histologic and histometric analysis

Samples of the transplant tissue were fixed in 4% formalin for 3 days and then decalcified with 5% EDTA(pH 8.0), dehydrated in ethanol, and embedded in paraffin. The central sections were reduced to a thickness of 5 μ m and then stained with hematoxylin and eosin(H-E). Light and polarized-light microscopy(Olympus BX50, Olympus Optical, Tokyo, Japan) were used for the histological analyses. The number of cells was counted using an automated image-analysis system(Image-Pro Plus, Media Cybernetics, Silver Spring, MD, USA). The formation and organization of Sharpey's fibers resembling tissue in the collagen were observed following Picrosirius staining.

4. Immunohistochemical analysis

For immunohistochemical analysis, sections were immersed in 0.3% hydrogen peroxide to block endogenous peroxidase activity, and then incubated with primary antibodies diluted in PBS (1:200-1:500). A human-specific mitochondrial antibody(mitochondrial ribosomal protein L11, hMito; Abcam, Cambridge, UK) diluted to 1:100 was used to confirm the origin of the human cells. Analysis of markers related to collagen formation was achieved using the following primary antibodies: collagen type I(Col I, Collagen I antibody; Abcam), collagen type III(Col III, Collagen III antibody; Abcam), and

hydroxyproline(hydroxyproline antibody; Bioss). A commercially available kit(Zymed SuperPicTure polymer detection kit, Zymed, Invitrogen, Carlsbad, CA, USA) was used to detect the antibodies according to the manufacturer's protocol. Slides were then counterstained with hematoxylin. Intensity of immunohistochemical staining at interested point was evaluated according to our previous study¹²⁾.

5. Effect of EMD treatment on soluble collagen and sulfated glycosaminoglycans(GAG) formation by hPDLSCs in vitro; Sircol collagen assay and Blyscan sulfated GAG assay

When the culture of hPDLSCs came to the subconfluent stage, hPDLSCs were cultured in medium with EMD at various test concentrations: 0, 100, and 200 $\mu\text{g}/\text{ml}$. After 5 days, the cell supernatants were collected by centrifugation at $260 \times g$ for 5 min for total soluble collagen formation. Total soluble collagen in the cell supernatant was measured using the Sircol collagen assay kit(Biocolour, Newtownabbey, UK). Briefly, a 200- μl aliquot of the supernatant was added to 1 ml of the dye reagent provided with the kit, and the mixture was incubated for 30 min at room temperature. After centrifugation at $9,300 \times g$ for 10 min, the separated suspensions were discarded and the remnant pellets were dissolved in 1 ml of the alkali reagent provided with the kit. The relative absorbance was then measured at 555 nm.

For the detection of the sulfated GAG by hPDLSCs the cell supernatants were collected by

centrifugation at $260 \times g$ for 5 min, and then 1 ml of the dye reagent was added to a 100- μl aliquot of the supernatant. The tubes were centrifuged at $13,400 \times g$ for 10 min and a dissociation reagent was then added(0.5 ml) to each tube. After ensuring thorough dissolution, the mixture was loaded onto 96-well plates, which were placed into a microplate reader and the absorbance was measured at 656 nm.

6. Statistical analysis

All experiments repeated 3 times and the data were presented as mean and standard deviation. Student's t test was used to analyze the statistical significance of differences between the EMD⁻/hPDLSC⁺ and EMD⁺/hPDLSC⁺ groups. One-way ANOVA was used to compare the soluble collagen and GAG among the three EMD concentration conditions(i.e., 0, 100, and 200 $\mu\text{g}/\text{ml}$). The statistical analysis was carried out using SPSS for Windows version 18.0(SPSS, Chicago, IL, USA). The cutoff for statistical significance was set at $P < 0.05$.

III. Results

1. Effect of EMD on the formation of cementum-like tissue, cementocytes, and Sharpey's fibers resembling tissue by hPDLSCs: a histological and histometric analysis of an in vivo model

The clinically using dose of EMD(30 mg/ml) was applied in vivo model, because the PDLSCs

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from human were used for transplantation. Histologic evaluation revealed that there was no any significant adverse effect or inflammatory reaction in both experiment groups.

Newly formed mineralized/cementum-like tissue was observed in association with the transplanted hPDLSCs along the HA/ β -TCP particles. Newly formed cementum-like tissue was present along the periphery of the HA/ β -TCP

carriers, and a high degree of mineralization was observed in both groups(Fig.1A-D).The new cementum-like tissue of both group was characterized by a highly mineralized collagen matrix and numerous cementocytes were embedded within the mineralized tissue. The histologic phenotype of newly formed cementum-like tissue was different from the bone like tissue showing rare lamella structure in line with

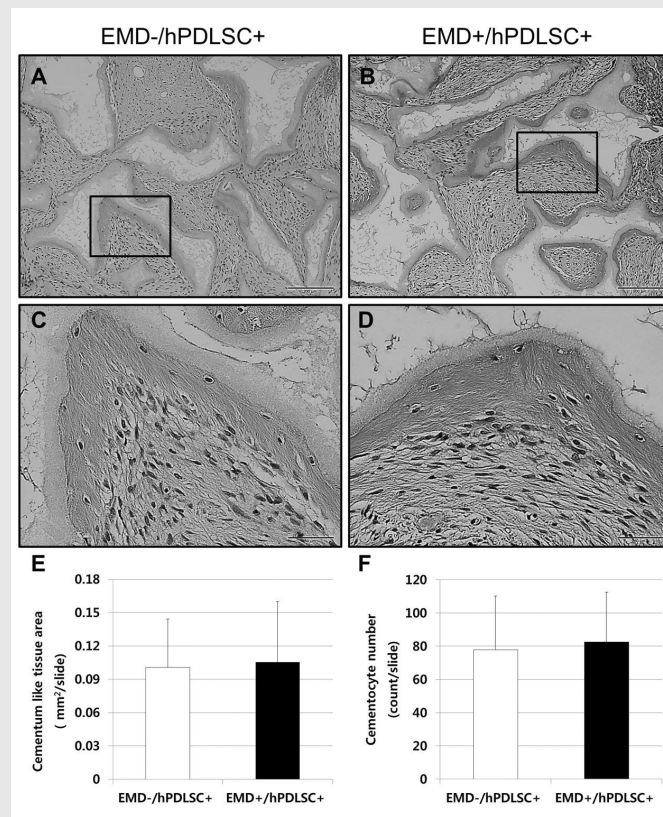


Fig. 1. Effect of enamel matrix derivatives(EMD) on human periodontal ligament stem cell(hPDLSC)-induced cementum formation and cementocyte proliferation in vivo. (A and B) Histology of new cementum formation was apparent around hPDLSCs seeded onto an untreated HA/ β -TCP carrier (EMD-/hPDLSC+ group) and an EMD-pretreated HA/ β -TCP carrier (EMD+/hPDLSC+ group) (original magnification= $\times 100$, scale bar=500 μ m). (C and D) Higher magnification view of the inset box in A and B; new cementum formation was confirmed around hPDLSCs seeded onto an EMD-untreated HA/ β -TCP carrier and hPDLSCs seeded onto an EMD-pretreated HA/ β -TCP carrier. Numerous cementocytes can be observed in the new cementum tissue (arrowheads; original magnification= $\times 400$, scale bar=100 μ m). (E) Histometric analysis revealed that the area of new cementum formation was similar in the EMD-/hPDLSC+ and EMD+/hPDLSC+ groups (no statistically significant difference). (F) Comparable numbers of cementocytes were found in the EMD-/hPDLSC+ and EMD+/hPDLSC+ groups (no statistically significant difference).

the previous studies¹⁷⁾. Interestingly, in the bone-like tissue, multinucleated osteoclast-like cells were regularly observed in relation to the osteoblast lining, however, in the formation cementum-like tissue, such appearances were seldom found. Also, the cell rich zone composed of well-organized collagenous ligament fiber like tissue and characteristic finding of histology produced by PDLSC was clearly observed in both groups¹³⁾. However, there was no significant histological difference according to the treatment of EMD in histologic analysis.

Likewise, the H-E staining revealed that the total area of newly formed cementum-like tissue was similar in the EMD-/hPDLSC⁺ and EMD⁺/hPDLSC⁺ groups and there was no statistically significant difference between groups(Fig. 1E)¹⁸⁾. A number of cementocytes were observed in the lacunae of the newly formed cementum-like tissue(Fig. 1C and D), and the total number of cementocytes was counted. The number of cementocytes was also comparable in the EMD-/hPDLSC⁺ and EMD⁺/hPDLSC⁺ groups without any statistically significant difference(Fig. 1F).

When microscopic observation under the Picrosirius stain the formation of Sharpey's fiber resembling tissue could be observed clearly(Fig. 2A and B). The Sharpey's fiber resembling tissue was well associated with new cementum-like tissue running into the PDL spaces mimicking histologic pattern shown in the natural periodontal tissue. The insertion of Sharpey's fiber resembling tissue into the cementum-like tissue originated from the collagenous tissues is

the typical finding in the regenerated mineralized tissue by hPDLSCs as reported in the previous study¹³⁾. It seems that longer healing period may have resulted a difference in cementum-like tissue formation. Inserted Sharpey's fibers resembling tissue were also frequently observed under the polarized light microscope(Fig. 2C and D). The total number of inserted Sharpey's fiber resembling tissue was measured using the polarized light images, and the numbers per unit area was significantly greater in the EMD⁺/hPDLSC⁺ group than in the EMD-/hPDLSC⁺ group($P<0.05$; Fig. 2E). In addition, the Sharpey's fiber in the EMD⁺/hPDLSC⁺ group appeared longer and progressed into the PDL space. This suggests that EMD has no significant effect on the formation of mineralized tissue but may provide a beneficial effect on the collagenous PDL fiber which has a pivotal role in tooth attachment.

2. Immunohistochemical analysis

Based on the histological analysis of new cementum-like tissue and fiber formation, immunohistochemical staining was performed to further understand the mechanism of cementogenesis and collagenous ligament fiber formation(Fig. 3). In previous study, cementogenic differentiation was preceded by collagenous ligament fiber formation¹³⁾. Therefore, the representative cementogenic makers were used: ALP for earlier differentiation and OCN for later stage. As a result, ALP showed slightly increased intensity in the EMD group but there was no

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difference in the intensity of OCN between the two groups. Positively stained cells against hMito in both experimental groups revealed that the newly regenerated cementum-like tissue is originated from the transplanted human cells. Col I and III which are distinctive collagen subtypes comprising the PDL were strongly expressed in the EMD treated group along with the histologic analysis which revealed increase in collagenous ligament fiber formation. This could be also

confirmed through hydroxyproline staining.

3. In vitro analysis of collagen synthesis : Sircol collagen assay, Blyscans GAG assay

Collagens and GAG are major components of connective tissue, and GAG interacts with collagen in life structure¹⁹⁾. Therefore, the effect of EMD on the regenerative potential of

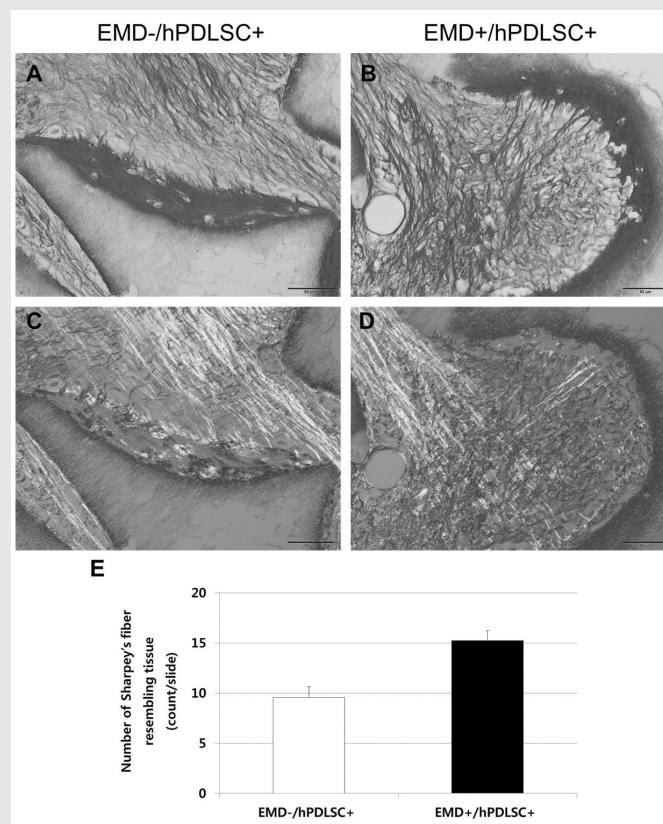


Fig. 2. Effect of EMD on hPDLSC-induced Sharpey's fiber resembling tissue in vivo. Sharpey's fiber resembling tissue was observed lining the surface of the carrier and running into the new cementum. (A and B) Newly formed Sharpey's fiber resembling tissue were confirmed around EMD-untreated HA/ β -TCP carrier cultured with hPDLSCs (EMD-/hPDLSC⁺ group) and an EMD-pretreated HA/ β -TCP carrier (EMD+/hPDLSC⁺ group) (arrowheads; original magnification = $\times 400$, scale bar = 100 μ m). (C and D) The polarized light microscopical images showed numerous insertion of Sharpey's fiber resembling tissue running parallel into the cementum tissue. (E) The number of new Sharpey's fiber resembling tissue was significantly greater in the EMD+/hPDLSC⁺ group than in the EMD-/hPDLSC⁺ group (*, $P < 0.05$).

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hPDLSCs regarding collagen and GAG production was assessed in vitro. Sircol collagen assay is an established evaluation tool for the measurement of collagen formation¹⁵⁾. The results

showed that collagen and GAG formation by the hPDLSCs was significantly enhanced by EMD in a dose-dependent manner(Figs. 4 and 5). Statistically significant differences in the degree of

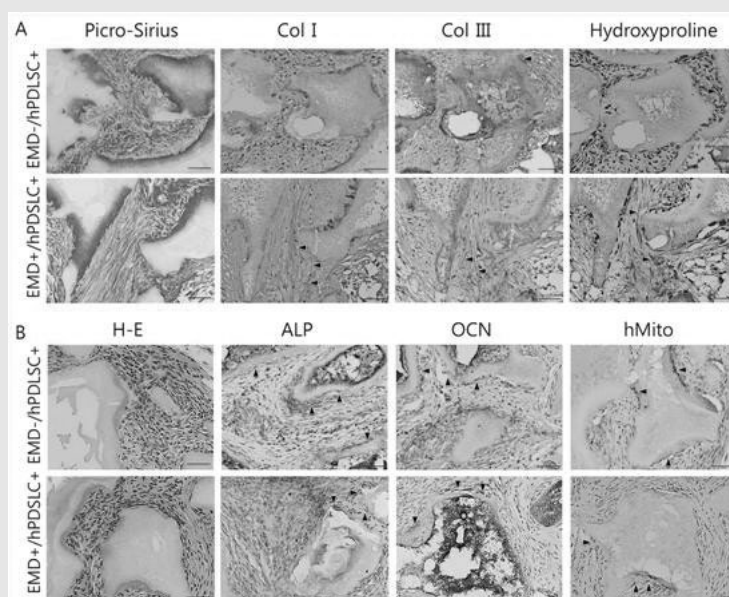


Fig. 3. Immunohistochemical staining. The retrieved specimen from the ectopic transplantation model were stained against various antigens including Col I, Col III, hydroxyproline, ALP, OCN and hMito. Picrosirius staining distinctively demonstrate the running of collagen fibers, especially in EMD treated group. Cells were comparably stained against other antigens in both groups.

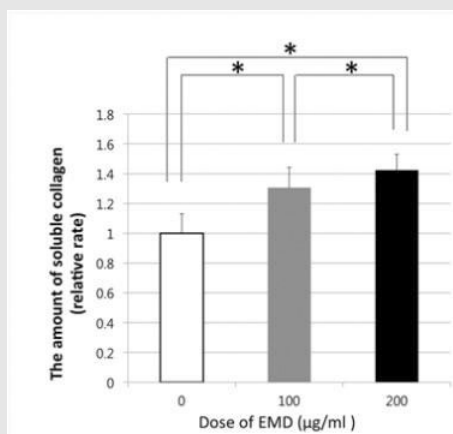


Fig. 4. Enhanced soluble collagen synthesis by EMD-treated hPDLSCs. The Sircol collagen detection assay revealed that EMD increased the production of soluble collagen by hPDLSCs in a dose-dependent manner (*, $P<0.05$).

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collagen and GAG formation were observed between each of the three EMD concentration groups (i.e., 0, 100, and 200 $\mu\text{g/ml}$).

IV. Discussion

The main function of PDLSC is the formation of cementum and collagenous ligament fibers responsible for the critical role of attachment. The periodontal regenerative effects of EMD have been well documented, but the cellular mechanisms are yet unknown. Based on the PDLSC technology, the results of the current study composed of in vitro and in vivo assays showed that EMD application had no effect on PDLSCs' cementogenic potential, but through enhancement of collagen and GAG formation, potential for formation of collagenous ligament fiber may be increased, which has a pivotal role in periodontal attachment. This may bring forth a

cellular mechanism of EMD's beneficial effect on the enhancement of periodontal regeneration.

The results of this study indicated that the area of new cementum-like tissue formation in vivo was comparable in the EMD/hPDLSC⁺ and EMD⁺/hPDLSC⁺ groups. It has been previously reported that EMD enhanced mineralization in association with an up-regulation of bone markers in human mesenchymal stem cells (hMSCs) in bone marrow during osteogenic differentiation,²⁰⁾ and it appears to significantly induce proliferation of hMSCs and early-stage osteoblast differentiation in a concentration-dependent manner.²¹⁾ The number of cementocytes was also similar regardless of the treatment of EMD on hPDLSCs. In the present study, the results showed an interesting revelation that EMD did not induce any difference in the formation of cementum-like tissue in vivo, which may be due to the relatively brief observation period of 8 weeks. As our previous study

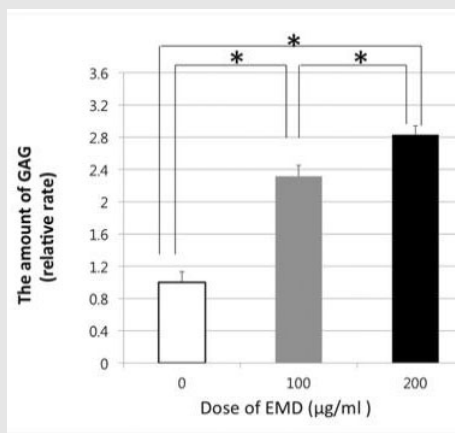


Fig. 5. Enhanced glycosaminoglycans(GAG) synthesis by EMD-treated hPDLSCs. The Blyscan sulfated GAG assay demonstrated that EMD increased the production of GAG by hPDLSCs in a dose-dependent manner (*, $P<0.05$)

showed¹³⁾, the healing of periodontal tissue follows a certain dynamic time process, and cementum formation may be preceded by the formation of collagens. We have demonstrated that the collagen fibers are regenerated first, then followed by the cementum formation induced by cementogenic signals. It was assumed that the formation of collagen fibers appear to be very important for the regeneration of cementum-like tissues in vivo. Therefore, it would be inappropriate to conclude that the treatment of EMD has failed to regenerate the cementum-like tissue, but it should be interpreted that the EMD has significantly induced the formation of collagen tissue such as Sharpey's fiber resembling tissue, which may be deeply involved in the formation of cementum in later stage and the functional attachment of Sharpey's fibers. Although we have followed only 8 weeks of healing period, the results may be different in the longer term of healing period, and the numerous clinical studies demonstrate that the healing period makes a very important difference in the maturation of the periodontal tissues²³⁾. Further studies are warranted to fully elucidate the effect of healing period on the regeneration of periodontal tissues treated by EMD.

The hPDLSCs were seeded onto a HA/ β -TCP carrier in an ectopic transplantation model using immunocompromised mice, which has been utilized in our previous studies to evaluate the biologic potential of transplanted cells^{23, 24)}. It was reported that the surface of HA/ β -TCP possess number of specially designed micropores and unique ionic microenvironment which is

favorable for cell attachment, proliferation and differentiation^{25, 26)}. Although the MBCP is known to exhibit osteoinductive capacity, previous studies reported that such osteoinductive effects were not observed in this model by using MBCP alone²⁷⁾, thus the negative control group was not included in this study design.

Additionally, we have confirmed that the formation of Sharpey's fiber resembling tissue was significantly up-regulated in the presence of EMD. To elucidate this phenomenon, we investigated the effect of EMD on soluble collagen formation using the Sircol collagen assay in vitro, and the results showed that soluble collagen formation by hPDLSCs in vitro was significantly enhanced by EMD in a dose-dependent manner. There are several reports of EMD enhancing the proliferation of PDL cells and increasing collagen production in vitro; thus, EMD appear to play an important role in periodontal tissue repair by the regeneration of collagen fibers^{12, 28, 29)}. Gestrelus et al. reported that EMD enhanced the proliferation of PDL cells and promoted mineralized nodule formation by PDL cells, but did not affect the migration or attachment in vitro¹²⁾. Cattaneo et al. showed that EMD enhanced the proliferation of PDL fibroblasts in a time-dependent manner²⁸⁾. In the present study, EMD significantly enhanced the formation of collagen fibers both in vivo and in vitro, and these results are in line with previous studies. The authors assume that the increased formation of collagen fibers and collagenous tissues in vitro and in vivo can play a critical role in the periodontal regeneration, and seem to be

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deeply involved in the formation of cement tissue as shown in our previous study¹³⁾ and further studies are warranted to clearly elucidate the underlying mechanism.

The results of this study also revealed that GAG formation by hPDLSCs was significantly enhanced by EMD in a dose-dependent manner. GAG, which vary in molecular size and characteristics, have many functions in the extracellular matrix, such as connective tissue matrix formation, regulation of cell growth, cell adhesion, and binding of growth factors³⁰⁾. Kirkham et al. reported that the GAG content of PDL is related to the maintenance of the unmineralized state of the PDL tissue³¹⁾, and it appears that GAG are very important for the stability of PDL tissue. Furthermore, EMD have also been shown to affect collagen fiber regeneration and the production of GAG^{19, 32, 33)}, and it was reported that EMD have the potential to modulate GAG in a manner consistent with the early regenerative period¹⁹⁾. Since collagen fibers and GAG play an important role in periodontal tissue regeneration, we could assume that the increased formation of collagen fibers and GAG by hPDLSCs play a critical role in the periodontal regeneration.

Up to date, research into the effects of EMD on hPDLSCs is lacking and controversial if any. Previous studies have shown that EMD could induce the regeneration and formation of acellular extrinsic fibercementum(AEFC)^{34~36)},

and it is well established that AEFC plays an important role in attachment during periodontal regeneration. However, some studies have demonstrated the EMD-induced formation of cellular intrinsic fiber cementum^{37, 38)}. In the present study, we could observe a number of cementocytes within the newly formed mineralized tissue, and it appears that the new cementum-like tissue formed under the treatment with EMD was mainly cellular, rather than acellular cementum. The present findings are thus more in line with those of the latter studies.

Collectively, the results of this study demonstrated that the EMD significantly enhanced the collagen formation by hPDLSCs, which may be deeply involved in the functional attachment of PDL. Although there was no difference of the cementum-like tissue formation according to the aid of EMD in 8 weeks, the results might be different in the longer term of healing period. These findings from the current study provide insights into the mechanisms underlying periodontal regeneration by hPDLSCs and a potential treatment strategy for periodontal defects.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported

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